Relation of Rhizobia to Alfalfa Sickness in Eastern Washington

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CONTENTS

Relation of Rhizobia to Alfalfa Sickness in Eastern Washington[1]

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INTRODUCTION

A condition, known locally as "alfalfa sickness," is prevalent in cutover forest and adjacent prairie areas of eastern Washington and northern Idaho. Alfalfa sickness, characterized by unthrifty, spindly, chlorotic plants, results in low alfalfa production (figure 1).

Alfalfa sickness exists in areas with recorded histories of good alfalfa production verified by the fact that some fields show good legume growth, as do isolated patches or even individual plants in otherwise unthrifty stands. Furthermore, the evidence of good growth along fence rows, the bottoms of hills, fill areas, burn rows, and road ditches indicates that good growth in the area is possible.

Examination of the roots of normal plants shows numerous well-formed, red-pigmented nodules, while examination of chlorotic plants shows malformed, nonpigmented nodules or the complete absence of nodules (figure 2). Sufficient nitrogen added to the unthrifty plants results in improved growth. This suggests an inadequacy in the legume-rhizobia symbiosis.

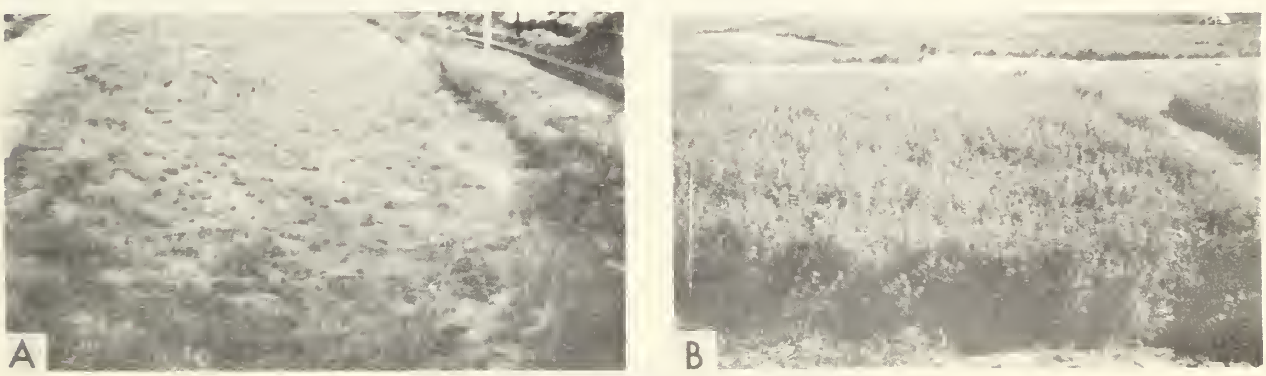

Figure 1.--A, Field of alfalfa showing: A, Alfalfa sickness. Small areas of healthy plants interspersed among plants showing abnormal growth; B, with healthy normal growth.

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[1] Contribution from the Soil and Water Conservation Research Division, Agricultural Research Service, U.S. Department of Agriculture, in cooperation with the Washington State Agricultural Experiment Station.

[2] Research soil scientists, ARS, USDA, Prosser, Wash. The authors are indebted to G. M. Horner and E. E. Cary, Soil and Water Conservation Research Division, Agricultural Research Service, U.S. Department of Agriculture, Pullman, Wash., for assistance in conducting this study.

Figure 2.--Roots of alfalfa plants with A, effective nodulation and B, ineffective nodulation.

PRELIMINARY INVESTIGATION

A preliminary experiment was begun in June 1961. Six plants showing typical symptoms of alfalfa sickness were taken from the field into the greenhouse. Enough soil was left on the roots to fill 6-inch pots. They were grown for 3 months without treatment. Growth during this time was poor. In September some of the plants were inoculated with an effective rhizobial strain, and within a short time these plants showed improved growth and color. This condition prevailed until April 1962 when growth and color of the uninoculated plants began to improve. Numerous healthy nodules were present on the roots of the uninoculated plants near the hole in the bottom of the pot (figure 3). Presumably, inoculation of these plants occurred via dust during an extremely windy period.

In these preliminary greenhouse studies, the increased good growth due to effective nodulation and nitrogen fixation furnished a lead to what was thought to be a primary cause of alfalfa sickness. This evidence led to the study of the relationship of rhizobia to the poor performance of alfalfa in the affected areas.

GENERAL CHARACTERISTICS OF THE ALFALFA AREA STUDIED

The area of study is located in the Latah-Rock Creek Soil and Water Conservation District, Spokane County, Wash., and in the adjacent Kootenai Soil Conservation District, Kootenai County, Idaho.

Alfalfa is grown in the area both on cutover forest soils and on the adjacent prairie soils. Alfalfa sickness is common on both, especially where erosion has been severe.

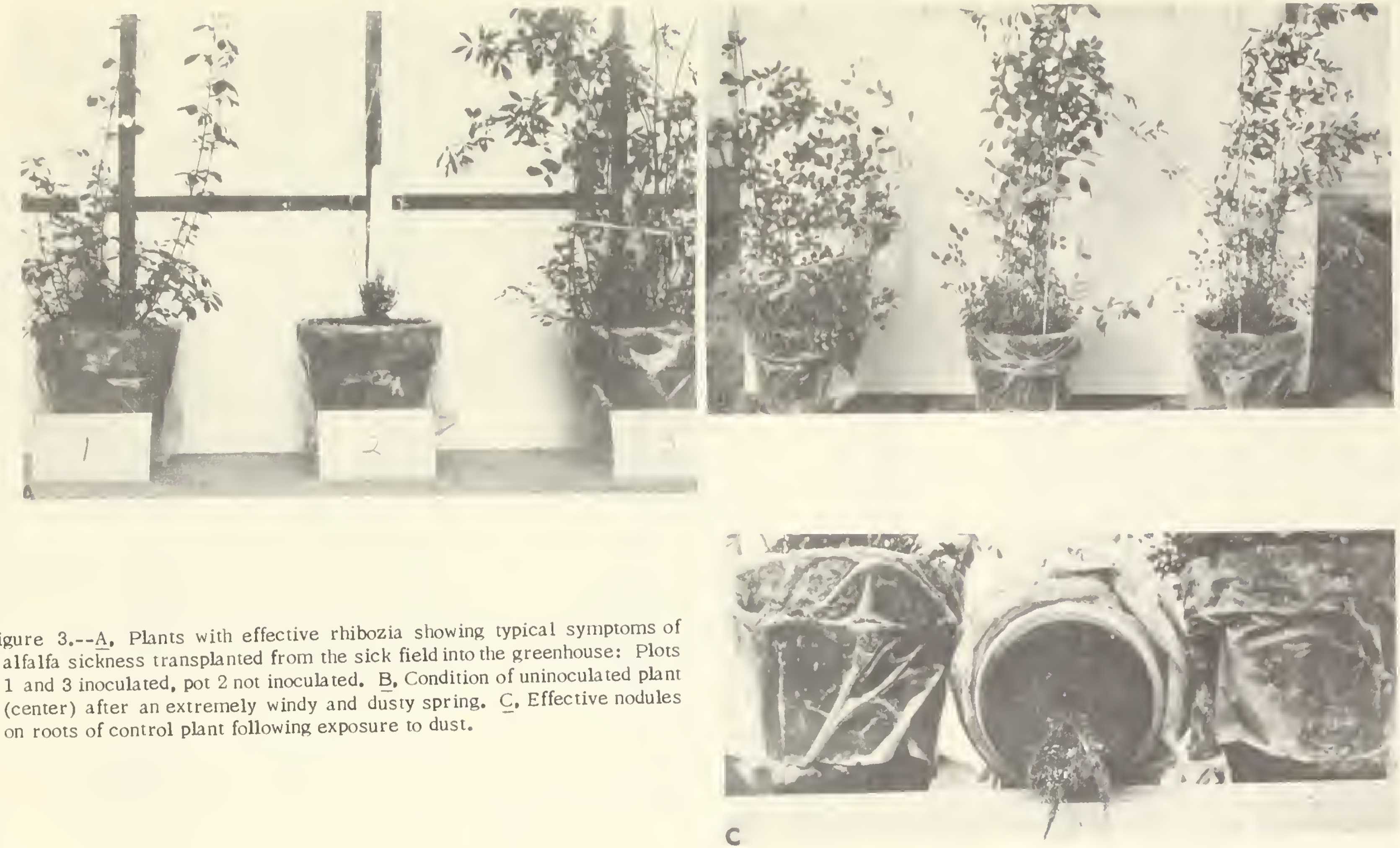

Figure 3.--A, Plants with effective rhibozia showing typical symptoms of alfalfa sickness transplanted from the sick field into the greenhouse: Plots 1 and 3 inoculated, pot 2 not inoculated. B, Condition of uninoculated plant (center) after an extremely windy and dusty spring. C, Effective nodules on roots of control plant following exposure to dust.

The soil of this study is classified as Freeman silt loam, a planosol, having well-developed textural horizons. The surface layer is low in organic matter and fertility, acid in reaction, eroded, and has generally poor physical characteristics. One morphological feature of this soil bearing directly on the growth of alfalfa is the presence of a well-developed B_2 horizon (bulk density 1.65, clay content 30 to 35 percent) which generally occurs within a depth of 2 feet.[3] This horizon restricts root and water penetration, resulting in extremely wet A horizons during winter and spring that become dry during July and August.[4] Frost heaving often aggravates plant growth in the spring.

Alfalfa sickness is not found exclusively on this soil; it has been observed in other areas on sandy loam soils not having well-developed textural horizons.

Performance of alfalfa varies from field to field in the alfalfa-sickness area. Growth, following the late spring seeding date (May) is usually normal. Variations in growth during this period seem to be conditioned mainly by variations of moisture and fertility. Alfalfa-sickness symptoms generally appear the following year in uneven patterns throughout the field. This problem may persist for several years with eventual recovery of the surviving plants.

EXPERIMENTAL PROCEDURES

Collection of Soil Samples

Bulk samples of the 0- to 7-inch layer of Freeman soil were obtained from the Tiede and Ramsey farms near Rockford, Wash. The soil from the Tiede location was taken from an area showing extreme alfalfa sickness, whereas that from the Ramsey location represents a mild case of the disorder. The samples taken at both locations were stored in the field-moist condition in plastic-lined burlap or canvas bags until used in greenhouse tests. This storage procedure was adopted to prevent contamination via dust and to prevent desiccation effects on soil microbial populations.

Greenhouse Pot Experiments

Several pot experiments were conducted in the greenhouse designed to study the effects of source of inocula, rate of inocula, and the effects of nutrient elements and liming materials on nodulation and plant growth. Because of the diversity of the experiments, the details of each experiment are discussed along with the experimental results. Where liming materials were added, they were incorporated in the soil prior to seeding and inoculation. Fertilizers were added as dry salts prior to seeding, or as Ashby's nutrient solution[5] without nitrogen. Commercial inoculum was applied as the dry powder; laboratory preparations were applied as a drench. Rhizobia were isolated from alfalfa nodules and stored using conventional methods[6].

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[3] Personal Communication, G. M. Horner, S.W.C., Pullman, Wash.

[4] Schwendiman, J. L. Mimeo., Report of Conference on Legume Sickness, Moscow, Idaho. 1959.

[5] Harris, J. O. Pipeline protective coating materials as growth substrates for soil microorganisms. Trans. Kans. Acad. Sci. 62: 42-46. 1959.

[6] Allen, O. N. Experiments in Soil Bacteriology. Burgess Publishing Co., Minneapolis 15, Minn. 1959.

RESULTS

Effectiveness of Rhizobia Isolated From Nodules of Sick and Healthy Alfalfa Plants

Rhizobium isolates cultured from nodules of sick and healthy plants from the alfalfa-sickness area, as well as other cultures of known effectiveness, were used as sources of inoculum in greenhouse experiments. Surface-sterilized Ladak alfalfa seeds were planted in pint cartons containing 400 g. of Freeman soil from the Tiede location. Upon emergence the stand was thinned to 5 plants per pot. The experiment was split into duplicated fertilized and unfertilized series. The fertilized series received 100 ml. of Ashby's nutrient solution from which the nitrogen was omitted. The yields of the plants and the numbers of nodules on the roots are given in table 1.

Table 1.--Effectiveness of rhizobial strains on alfalfa grown on Freeman silt loam from the Tiede farm location [1]

Inoculum treatment	Unfertilized [2]		Fertilized [2]	
	Nodules per 10 plants	Dry wt. per 10 plants	Nodules per 10 plants	Dry wt. per 10 plants
		G.		G.
Uninoculated	13	0.25	7	0.17
Beltsville strains:				
$3DO_a23$	1,182	7.12	805	10.60
$3DO_a$	1,010	12.18	933	10.10
$3DO_a24_a$	817	10.91	730	12.67
$3DO_a21_a$	951	9.60	853(9)	12.80(9)
$3DO_a20_a$	468(8)	4.12(8)	875	9.55
$3DO_h13$	589	5.07	618(9)	8.50(9)
Commercial strains:				
A	573	5.20	493	13.19
B	468	4.40	361	8.20
Field isolates from sick plants:				
A	36	0.17	40	0.40
B	30	0.17	14	0.27
C	11	0.09	3	0.10
D	23	0.09	20	0.10
L_2	1	0.10	7	0.10
K_{1-3}	9	0.11	14	0.16
Field isolates from healthy plants:				
E	934	10.04	1,172	13.55
F	741	8.91	867	14.84
G	1,534	9.56	1,096	14.76
Prosser 4	1,029	5.76	1,382	11.84
Prosser 9	1,477	9.38	848	11.17

[1] Planted February 12, 1962; harvested May 1, 1962.

[2] Numbers in parentheses refer to number of plants in two cartons.

The plants not receiving inoculum, and those inoculated with rhizobia from sick plants, were small and chlorotic and showed typical alfalfa-sickness symptoms (figure 4). On the other hand, plants inoculated with the Beltsville strains, the commercial strains, and isolates from healthy plants grew vigorously and normally. The results of another experiment not reported here showed that 10 additional isolates taken from sick plants were also ineffective in supplying the nitrogen needed by plants.

A comparison of the plant yield data for the fertilized and unfertilized series indicates that the plants inoculated with effective rhizobia benefitted from the added nutrients, whereas the others did not.

Nodules were present on most plants. Where plants were green and growth vigorous, nodules were normal, pigmented, and numerous. Where plants lacked vigor and were chlorotic, nodules were small, brownish, malformed, nonpigmented, and few in number.

These data indicate that in this soil there are strains of rhizobia capable of forming nodules but completely ineffective in supplying adequate nitrogen to the plant.

To eliminate environmental and nutritional factors that the soil from the sick alfalfa area might have on the rhizobia, another experiment, using sterile sand as the rooting medium, was conducted. One hundred mililiters of Ashby's nutrient solution (without nitrogen) was added to supply nutrient elements other than nitrogen. The ineffective rhizobial strains used were isolated from nodules of chlorotic plants in another experiment. An effective strain was included in this experiment also. Five cartons of sand plus nutrient solution for each rhizobial strain were seeded and inoculated. After 1 month, the roots of plants in one pot from each group were inspected and found to be nodulated. Of the ineffective strain series, one-half of the pots were then reinoculated with an effective strain. Similarly, one-half of the pots initially inoculated with an effective strain were then reinoculated with an ineffective strain.

The results given in table 2 are similar to those obtained when soil from the Tiede location was used. The plants initially inoculated with ineffective rhizobia that were not reinoculated with an effective strain were small and chlorotic throughout the experiment. Those inoculated initially with an effective strain grew vigorously and appeared normal. After 1 month, when effective rhizobia were added to the pots that had initially received ineffective rhizobia, there was a rapid

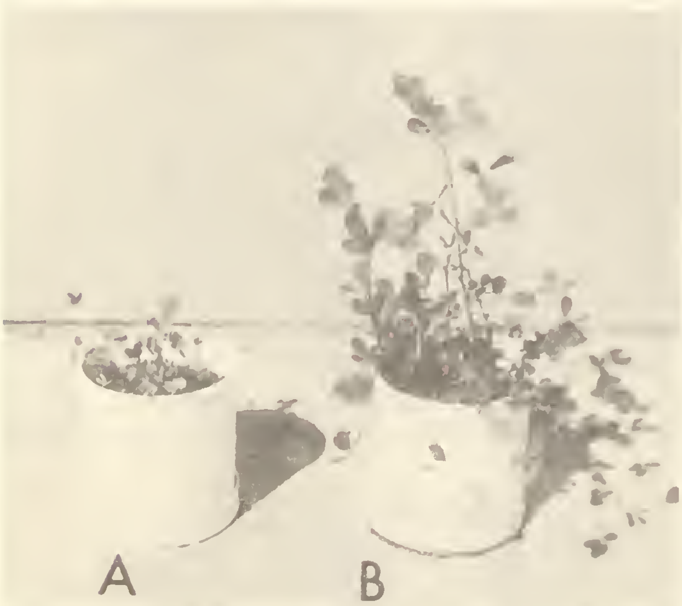

Figure 4.--Plant inoculated (A) with ineffective rhizobia and (B) with effective rhizobia.

Table 2.--Effect of successive inoculations on the yield of alfalfa grown in sand culture

Inoculation treatment	Dry Weight 10 Plants[1]
	G.
Effective strain 2/14/62 Ineffective strain 3/14/62	6.56
Effective strain 2/14/62 Sterilized ineffective strain 3/14/62	6.20
Ineffective strain 2/14/62 Effective strain 3/14/62	4.35
Ineffective strain 2/14/62 Sterilized effective strain 3/14/62	0.82

[1] All plants harvested April 14, 1962.

growth response. This indicated that the effective organisms had infected the roots and were actively fixing nitrogen. Reinoculation of the plants that had initially received effective rhizobia with an ineffective strain did not affect the growth or final yield of the plants. Thus, poor strains remained ineffective and good strains remained effective for the duration of the experiment. Furthermore, the results indicate that the relative effectiveness of the two different strains of rhizobia is not related to the physical and nutritional properties of the soil from which they were isolated.

Prior infection with ineffective rhizobia did not appear to inhibit reinfection with effective rhizobia. At the time of reinoculation, nodules were present on the roots of the plants initially receiving ineffective rhizobia. At the end of the experiment, however, the roots of the plants reinoculated with effective rhizobia held numerous large, well-pigmented nodules. The addition of sterilized suspensions of either effective or ineffective strains to any of the systems did not affect the performance of either rhizobial strain. Thus, the differential ability of the different strains to fix nitrogen in symbiosis appears to be an inherent feature of the organisms themselves.

Effect of Number of Added Rhizobia on the Yield of Alfalfa

The response of alfalfa in some of the alfalfa-sickness area soils to effective rhizobia inoculum and the ability of some native indigenous rhizobia to nodulate, but not fix nitrogen, prompted an investigation to see how many efficient rhizobia per gram of soil were necessary to nodulate and fix nitrogen in alfalfa planted in this soil.

The results of a preliminary experiment indicated that the required inoculum dosage was somewhere between 12,000 and 120,000 organisms per gram of soil (figure 5) for the soil from the Tiede location, and that inoculation had no effect on growth on the soil from the Ramsey location.

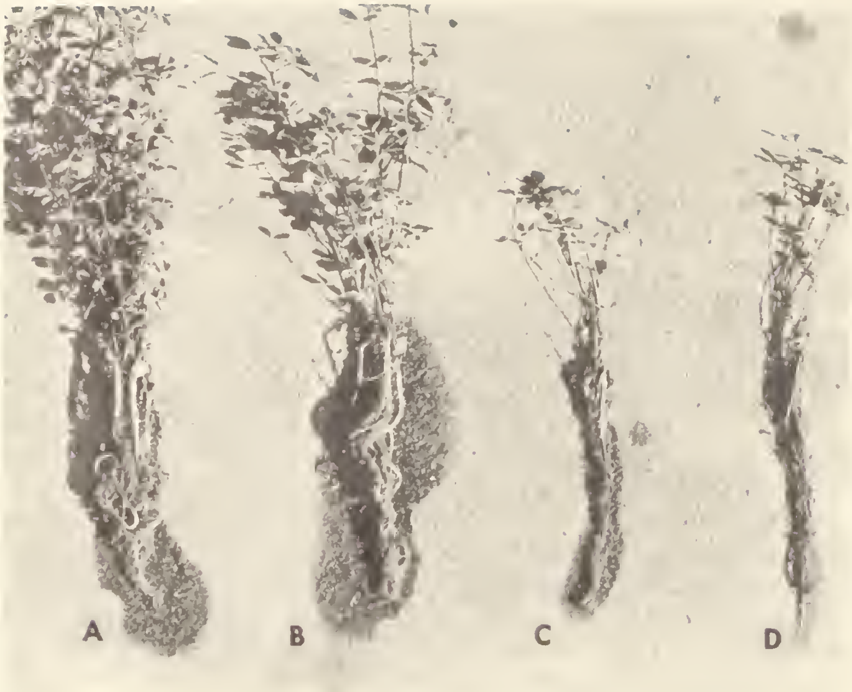

Figure 5.--Effect of rhizobial numbers on alfalfa planted in alfalfa sickness soil: A, 120,000; B, 12,000; C, 1,200; and D, 120 organisms added per gram of soil.

A second experiment was conducted to determine a lower dilution endpoint and to determine the effect of nutrient elements on inoculum levels required. From the Tiede and Ramsey locations 400 g. of soil were added to pint cartons. The pots were seeded with 10 surface-sterilized Ladak alfalfa seeds which, after germination and emergence, were thinned to 5 plants per pot. After seeding and prior to covering the seeds, known quantities of effective rhizobia strains were drenched over the seeds. The number of organisms added was determined by serial dilution and ranged from zero to 35,000 organisms per gram of soil. A fertilized series, as well as an unfertilized series, was included on both soils at all levels of inoculum. The nutrients were added to the fertilized series as 100 ml. of Ashby's nutrient solution (without nitrogen).

The results given in table 3 indicate that for the unfertilized series on the Tiede location soil 35,000 organisms per gram of soil gave maximum plant yield, although definite yield responses occurred at lower dosages. Fertilization resulted in higher yields and response to smaller dosages of inoculum than for the unfertilized series; a tenfold yield increase resulted from adding 35 organisms per gram of soil. No inoculation response was noted on soil from the Ramsey location although a definite response to fertilization was obtained.

A Survey of the Rhizobia Status of Fields in the Alfalfa-sickness Area

Results of some of the previous experiments indicate that the soil from the Tiede location contains few effective rhizobia. In almost all cases nodules were formed without inoculation, but poor plant growth showed that little, if any, nitrogen was fixed. Subsequent inoculation with large numbers of these ineffective organisms did not increase the growth and vigor of the plants. A survey of the alfalfa sickness area was conducted, therefore, to determine the extent of this condition.

Table 3.--Effect of number of added rhizobial cells on yield of alfalfa grown on fertilized and unfertilized alfalfa-sickness soils

No. of introduced R. meliloti cells (per g. soil)	Dry weight of tops [1]			
	Unfertilized		Fertilized [2]	
	Tiede Location	Ramsey Location	Tiede Location	Ramsey Location
	G.	G.	G.	G.
35,000	2.02	3.70	4.35	4.84
3,500	1.22	2.42	2.57	4.67
350	[3] .59	3.61	1.69	5.56
35	.23	2.85	2.16	5.75
3.5	.22	3.07	.22	3.08
---	.22	2.65	.20	5.14
nil	.27	2.31	.23	4.74

[1] Fourteen weeks' growth. Total weight from two pots.
[2] 100 ml. Ashby's nutrient solution (minus nitrogen) added.
[3] Two plants in one pot showed response.

A particular area lying to the southwest, south, southeast, and northeast of Mica Peak, Spokane County, Wash., was considered a problem area. Soil samples of approximately 500 g. were collected from the plow layer of each of 60 fields within the area. The samples were stored moist in plastic bags. To prevent cross contamination of samples, the sampling implements were disinfected prior to sampling each field. Three samples from the Irrigation Experiment Station at Prosser, representing an area of good alfalfa production and as having no particular inoculation problems, were included for comparison.

From each sampling location 400 g. of soil encased in polyethylene bags, were placed in pint containers, fertilized with 50 ml. of Ashby's nutrient solution (without nitrogen), and seeded with surface sterilized Ladak alfalfa seeds. After germination and establishment the plants were thinned to five per pot. After 14 weeks the plants were evaluated for growth, dry weights of forage, and condition of nodules.

The results from the 60 sampled fields are listed in table 4. The results of this sampling indicate that effective indigenous alfalfa rhizobia are scarce in much of the alfalfa-sickness area. In only 16 of the 60 fields sampled were there ample effective rhizobia which infected 80 percent or more of the plants. No effective rhizobia were found in 53 percent of the 60 field samples.

All of the plants, with the exception of those from one soil sample, had nodules on their roots. Nodules on the roots of the green plants were not always pink and normal in appearance; in a few samples vigorous plants were nodulated with ineffective rhizobia. In these cases the soil had a high nitrate-nitrogen content which furnished adequate nitrogen for the plants. Nodules on the roots of the chlorotic plants, however, were invariably abnormal, malformed, and lacked pigmentation. In contrast, all the plants grown on the three soil samples from the Prosser area grew normally and were well nodulated with effective rhizobia.

Since this experiment was of short duration, it is not known if additional plants eventually would have become infected with effective native rhizobia had the experiment been prolonged. In those pots where only part of the plants were chlorotic, i.e., where the soil contained a few effective organisms, there is a possibility that additional plants would have recovered. Under

Table 4.--A survey of the status of the rhizobia in soils of the alfalfa-sickness areas

No. of normal plants in carton[1]	No. of samples	Percent of total	Average dry wt. of tops
			G.
Samples from alfalfa-sickness area:			
0	32	53.3	0.50
1	7	11.7	.45
2	3	5.0	.91
3	2	3.3	.72
4	2	3.3	.88
5	14	23.3	1.66
Samples from Prosser, Wash.:			
5	3	---	1.77

[1] Five plants were grown in each sample. Fertilization consisted of 50 ml. of Ashby's nutrient solution (minus nitrogen). The growth period was 14 weeks.

field conditions where healthy and sick plants are interspersed, chlorotic plants eventually become well nodulated and recover, if they survive long enough. On the other hand, where all the plants were chlorotic after 14 weeks, it is unlikely that their condition would have improved with time. Chlorotic plants grown in the greenhouse under similar conditions for almost 1 year did not recover unless inoculated with effective rhizobia.

The results obtained from this survey are surprising because alfalfa has been a major crop in the area for many years. Indeed much of the area has probably received commercial inoculum sometime in the past when alfalfa plantings have been established. The histories of the sampled fields are not known at this time; hence, the data cannot be broken down to determine if previous inoculations may have affected the results of this experiment.

Effects of Nitrogen Fertilization on Nodulation and Yield of Alfalfa Grown on Soil From the Alfalfa-Sickness Area

Sick alfalfa responds to applied nitrogen in the field, especially at high rates of applications This indicates that less than adequate nitrogen fixation takes place in the plant, due to either no nodule formation, or to formation of ineffective nodules.

An experiment was conducted to ascertain the effect of nitrogen fertilization on the growth and nodule formation by alfalfa grown on a sick alfalfa soil. Prior to seeding Ladak alfalfa, urea was added to 400 g. of soil from the Tiede location. It was added in solution at rates calculated to supply from 0 to 640 pounds of nitrogen per acre. Blanket applications on a per-acre basis equivalent to 1 ton CaO, 200 lb. P, 100 lb. K, 40 lb. S, 3.5 lb. B, and 0.7 lb. Mo were incorporated previously into the soil samples, which were set up in replications of four, one-half of which were not inoculated with commercial inoculum.

Results of this experiment (table 5) indicate the inoculation response noted previously in other experiments. Plants growing in the uninoculated series fared poorly except under increasing

Table 5.--Effects of nitrogen on nodulation and yield of alfalfa grown on Freeman silt loam from the Tiede location[1]

Urea nitrogen rate lb./acre	Inoculated		Uninoculated	
	Nodules per 10 plants	Dry wt. per 10 plants	Nodules per 10 plants	Dry wt. per 10 plants
		G.		G.
0	386	4.98	39	0.51
10	727	4.85	45	.81
20	742	4.80	39	.67
40	691	6.12	142	.92
80	583	6.20	86	.95
160	520	5.10	102	1.65
320	429	4.15	160	2.56
640	269	3.90	61	4.86

[1] Planted March 27, 1962; harvested May 14, 1962.

rates of nitrogen. Low nitrogen rates exerted only slight influences on plant growth and only at high rates of fertilization did yields tend to approach those obtained with inoculation. However, in the small containers used, plants receiving nitrogen applications were vigorous and green early in the growth period, but tended to become slightly yellow and chlorotic at the end of the experiment. This effect was especially pronounced at low rates of nitrogen. In the inoculated series, yield tended to increase slightly at 40 and 80 pounds of nitrogen per acre, and then decrease progressively at higher rates. At the two highest rates used, nitrogen fertilizer decreased alfalfa yields to values that were lower than where no nitrogen was applied.

In the uninoculated series, nodules of ineffective rhizobia were formed on the roots of all plants. Moreover, rates of nitrogen from 40 to 320 pounds per acre increased nodule formation, probably as a result of larger and more extensive root systems. In the inoculated series, nitrogen fertilizer applied at rates up to 40 pounds per acre stimulated nodule formation. Higher rates of fertilization, however, progressively decreased the number of nodules formed, until at the highest rate used, fewer nodules were present than when no nitrogen was added. Thus, the effect of nitrogen fertilizer on nodule formation parallels the effect noted on yield.

Effects of Liming Materials and Gypsum on Nodulation and Alfalfa Yield

Applications of lime to soils in the alfalfa-sickness area often result in increased plant growth.[7] An experiment was conducted to determine the effects of dolomite, calcium carbonate, calcium oxide, and gypsum on nodulation and plant growth by alfalfa grown on soil from the Tiede location. Each of the amendments was added at rates equivalent to 1/2 and 5 tons of calcium carbonate per acre to 400 g. of soil. The experiment was split into an inoculated and an uninoculated series with two replications of each treatment. Inoculation was with a source of rhizobia known to be effective.

[7] Harder, R.W., Anderson, G.R., and Brackney, C.T. The influence of lime on the growth of alfalfa in northern Idaho. Idaho Agr. Res. Prog. Rpt. 67, 1962.

The data given in table 6 indicate a marked growth response to inoculation for all levels and sources of amendments. In the inoculated series, the highest rates of the liming materials resulted in the highest yields, whereas the gypsum applications resulted in no higher yields than did the inoculated control. The additional growth resulting from applying the liming materials probably resulted from the effect of increased pH on the availability of nutrients, or the effect of pH on the efficiency of nitrogen fixation by the rhizobia. In the uninoculated series none of the amendments affected plant growth to a measurable degree, thus indicating that liming does not alter the ability of native rhizobia to fix nitrogen.

Table 6.--Effect of calcium and magnesium materials on nodulation and alfalfa yield [1]

Treatment [2]	Soil pH	Inoculated		Uninoculated	
		Nodules per 10 plants	Dry wt. per 10 plants	Nodules per 10 plants	Dry wt. per 10 plants
			G.		G.
Control	5.4	111	1.52	23	0.19
Dolomite, high rate	6.4	139	3.00	10	.18
Dolomite, low rate	5.5	134	1.69	15	.17
$CaCO_3$, high rate	7.5	147	2.85	4	.10
$CaCO_3$, low rate	5.7	164	2.35	7	.11
CaO, high rate	7.7	270	4.41	50	.44
CaO, low rate	5.6	127	2.45	7	.11
Gypsum, high rate	4.9	72	1.84	22	.30
Gypsum, low rate	5.3	52	1.71	17	.21

[1] Planted November 22, 1961; harvested February 12, 1962.
[2] Low and high rates are equivalent to 1/2 and 5 tons of $CaCO_3$ per acre.

The highest rate of calcium oxide increased the numbers of nodules on the roots in both the inoculated and uninoculated series. Gypsum, on the other hand, depressed nodulation in the inoculated series; however, these nodules were larger and had better color than those for any of the other treatments.

DISCUSSION

Nodulation of alfalfa plants growing in the alfalfa-sickness area is as variable as the general growth and vigor of the plants themselves.

It is conceivable that the condition of each is responsible for the variability of the other, although the experiments reported herein indicate that the variability of nodulation is more directly responsible for the variability of growth.

Plants in the field that are unthrifty, spindly and chlorotic in appearance--symptoms identified with alfalfa sickness--are generally without root nodules or have nodules that are malformed, brownish, and nonpigmented. Conversely, plants exhibiting thriftiness and vigor are generally well-nodulated with well-shaped, pigmented, fingerlike protuberances or clusters of such, which are characteristic of effective rhizobia capable of fixing nitrogen in symbiosis with the plant.

Nitrogen applications to sick alfalfa plants, resulting in increased growth, substantiates either the absence or the ineffectiveness of rhizobia, or suggests the unavailability of some essential elements necessary for symbiotic nitrogen fixation within the plant environment.

Recovery after several years of field plantings having sporadic patches of healthy green plants indicates the presence of some effective rhizobia in the field system. A possible explanation for such recovery might be the distribution of organisms in ever-expanding radii from a loci of effective rhizobia, probably spreading along intertwining root systems.

In the present study, alfalfa-sickness symptoms were expressed in plants grown in the greenhouse on soils taken from the alfalfa-sickness area. The gross symptoms of the shoots of the plants were identical to those in the field, for example, unthrifty, spindly, and chlorotic. The root systems and nodules were likewise identical in condition and appearance to those from the sick field plants.

Only in rare instances were nodules not formed on root systems of alfalfa by the indigenous rhizobia of the soil in the greenhouse experiments. The nodule numbers were usually low and in most experiments contained ineffective rhizobia. However, some soil samples did contain very effective native rhizobia comparable to commercial strains in forming nodules and in fixing nitrogen symbiotically.

Inoculation with effective strains of Rhizobium meliloti produced well-nodulated alfalfa plants which grew vigorously as contrasted with those which, depending on native rhizobia, were poorly nodulated and low yielding. Strains from several sources known to be effective proved highly capable of producing nodules and fixing nitrogen.

Numbers of added effective rhizobia necessary to nodulate and fix nitrogen successfully varied with soil samples from different locations. Plants on some soils did not respond to inoculation because adequate effective indigenous rhizobia were present. On soils lacking adequate effective rhizobia, 35,000 organisms per gram of soil were needed for optimum yields. A minimum number of 3,500 organisms per gram of soil was required for healthy growth, but yields were only approximately one-half those of the high inoculum rate. Fertilization with all essential elements for alfalfa growth, except nitrogen, reduced the required minimum number to as low as 35 organisms per gram of soil. Yields under this treatment were comparable to those under 35,000 organisms unfertilized. However, yields with the highest inoculation rate under fertilization were double those of any other treatment. Fertility treatments on the soil samples not responding to inoculation greatly increased yields over those without such treatments.

Nodules formed by ineffective strains did not appear to inhibit nodulation by effective rhizobial strains.

Absence of nutritional elements in the soils that might be related to the ineffectiveness of a strain to fix nitrogen symbiotically was not demonstrated. Ineffective strains remained ineffective under optimum conditions for nodulation and nitrogen fixation. However, it should not be misconstrued that degrees of efficiency are not apparent among rhizobial strains, even poor strains.

The results of the partial survey of the alfalfa-sickness area soils revealed that rhizobia were present in almost all of the fields sampled, but that the ineffective organisms greatly outnumbered the effective ones. This situation is not unique to this area, as reports of rhizobia varying in effectiveness are well documented in the literature.[8] However, the necessity of reintroducing effective rhizobial strains into the area is evident, and could be accomplished through conscientious inoculation practices.

The limited experiments with lime and nutrient elements strongly suggest the involvement of nutritional factors in the problem. Previous work by others has shown nutritional responses on alfalfa in the area.[9]

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[8] Erdman, L.W. Legume Inoculation: What it is--what it does. U.S. Dept. Agr. Farmers Bul. 2003, 16 pp. 1959.

Mulder, E.G., and W. L. Van Veen, Effect of pH and organic compounds on N-fixation. Plant and Soil 13: 89-112. 1961.

[9] See footnote 7.

Variations in moisture content and the physical structure of the soil further complicate the situation.

Despite the obvious lack of answers to many of the questions on alfalfa sickness, the prospects for the successful culture of alfalfa in the alfalfa-sickness areas are encouraging.

SUMMARY

Alfalfa grown in soils from an alfalfa-sickness area was found to be poorly nodulated in greenhouse pot experiments. As a result, the plants exhibited nitrogen-deficiency symptoms and grew poorly. These symptoms were identical to those characterized as typical of alfalfa sickness in field plantings.

Inoculation of the soils with effective strains of Rhizobium meliloti gave well-nodulated plants which grew vigorously. Nodules on roots of these plants were well formed and pigmented in contrast to those from the unthrifty, chlorotic plants.

A soil sampling survey from the alfalfa-sickness area revealed a low incidence of good effective native rhizobial strains in some soils of the area studied, thus indicating that the scarcity of effective rhizobia may be limiting alfalfa growth.

Inoculation levels up to 35,000 organisms per gram of soil were necessary for optimum growth in unfertilized soil. Fertilized soils required only 35 organisms per gram of soil for effective nodulation and healthy growth, but maximum growth was attained under the high level of 35,000 organisms.

High rates of nitrogen are required under ineffective nodulation to equal yields of alfalfa inoculated with effective rhizobial strains and no nitrogen additions.

Alfalfa growth was influenced favorably by lime, and nutrient elements. Nodulation was improved under these treatments, also.